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Primer

Immunological imprinting: Understanding COVID-19

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SUMMARY

Immunological imprinting generically refers to the effects prior exposures have on subsequent immune responses to, and eventually protection against, antigenically related viruses. Here, Koutsakos and Ellebedy explain different concepts and terms around imprinting and the fundamental immunological principles behind it. They also discuss the potential role imprinting may have in the context of COVID-19 vaccines.

BASIC CONCEPTS OF IMPRINTING

In experiments done in the 1930s, young geese were found to form an attachment to moving objects they encountered at the very early stages of life after hatching, whether these were their mother, the experimenter himself, or inanimate objects. The authors described this phenomenon as imprinting, a term now used in psychology to describe the learning of behaviors by exposure during a critical period in early life.

In a manner somewhat like juvenile geese, the naive immune system, upon primary exposure to a virus, becomes “imprinted” by this exposure. It learns to recognize antigenic determinants in that virus and generates memory cells that respond to antigenically related viruses in the future. Indeed, humans maintain a life-long pattern of heightened antibody reactivity against influenza viruses that circulated during their childhood.¹ These patterns are referred to as antigenic imprinting, immunological imprinting, immune imprinting, or original antigenic sin (OAS), the latter being an often-misinterpreted term. The term “immunological imprinting” has become popular in viral immunology following seminal epidemiological studies that associated the subtype of influenza A virus encountered in early life to susceptibility toward severe influenza infection later in life. Additionally, there are numerous examples of how prior exposures to influenza shape subsequent immune responses to vaccination and infection and, in some cases, potentially protection from disease following re-infection. Similar questions are being posed in the context of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Do previous exposures to human coronaviruses (hCoVs) impact immune responses and susceptibility to SARS-CoV-2 infection and/or vaccination or COVID-19 severity? Does a previous exposure to one SARS-CoV-2 variant affect the immunological and/or clinical outcome following re-infection with an antigenically drifted lineage?

Any such effects of exposure history, so-called “immunological imprinting”, can be broken down to basic principles of the adaptive immune system, namely immunological memory, and cross-reactivity. From there, exposure history may or may not

have (positive or negative) immunological effects and subsequent implications for vaccine design or viral epidemiology. However, the formulation of such hypotheses, the interpretation of experimental evidence, and the synthesis of unified models are crucially hindered by inconsistent use of ambiguous terminology. Clear and consistent terminology and interpretation of findings in the context of basic immunological principles will be imperative in disentangling exposure history’s implications for infectious diseases, including COVID-19.

THE ORIGINAL ANTIGENIC “SIN”

Serological analyses of different age groups at baseline and following vaccination by Francis and others from as early as the 1940s led to two important observations: (1) individuals of all age groups had the highest antibody titers to strains they would have encountered in childhood and (2) some individuals had strong immune memory recall responses toward a previously circulating (historic) influenza A strain not included in the vaccine. This response was only observed in individuals born around the time of circulation of the historic strain but not in younger individuals born later that had not been exposed to that strain. These observations led Francis to propose that the first (*original*) strain encountered in childhood leaves a lifelong *antigenic bias*. Although subsequent exposures broaden the reactivity spectrum to encompass newer strains, reactivity toward the original strain is maintained at higher levels than reactivity toward newer strains. Francis’ use of the word “sin” is frequently assumed to suggest a negative consequence of this phenomenon. However, in the serological analyses mentioned above, antibody responses to the vaccine antigen were similar across age groups and not limited by the recall of antibodies to the original strain. Importantly, Francis proposed that OAS could be exploited for our benefit and become a “blessing” with the right vaccine formulation (see Monto et al.² for detailed discussion).

The literature on OAS, inherently focused on influenza, is often seemingly contradictory. This results from inconsistent definitions of “OAS” and different methodologies to measure



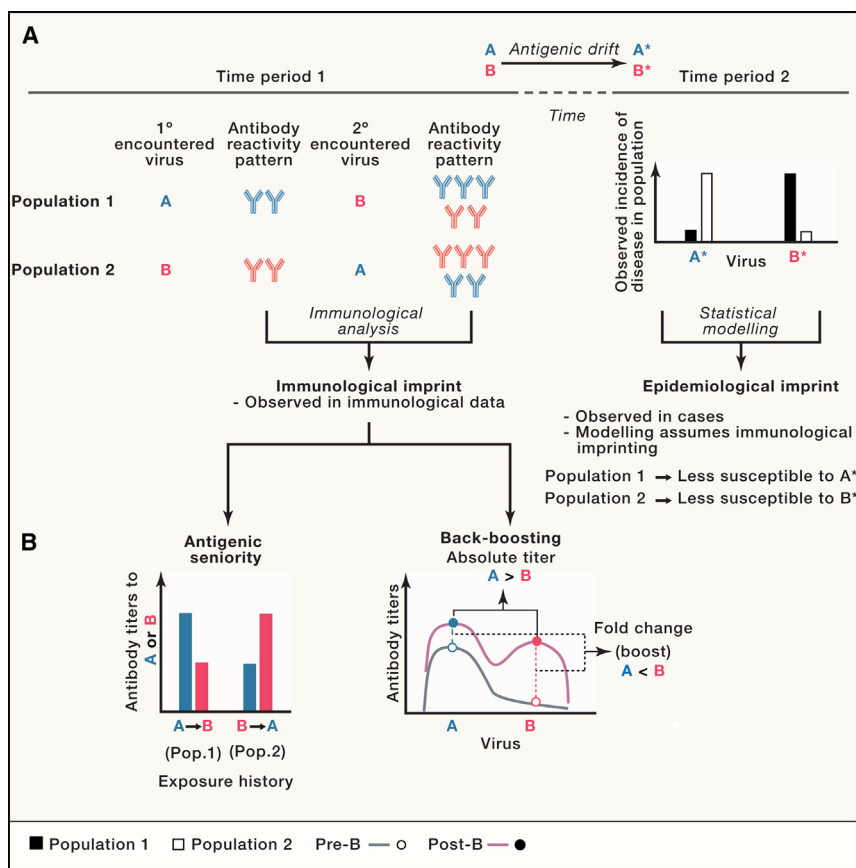


Figure 1. Immunological and epidemiological patterns observed across populations with different exposure histories

(A) Populations with different exposure histories may present with different antibody reactivity patterns toward antigenically related viruses. This immunological imprint can be observed in immunological data, like serological analyses. If these immunological differences translate into differences in susceptibility, the differential incidence of disease may be observed between populations over time. This “epidemiological imprint” can be observed in case distributions and is typically inferred from statistical models that link most likely exposure histories to the observed epidemiology. (B) Two commonly observed immunological patterns are antigenic seniority (observed in cross-sectional data) and back-boosting (observed in longitudinal data).

enza disease, assuming this stems from immunity toward antigens encountered earliest in life, which would be similar for a given birth cohort. In that context, imprinting referred to a parameter incorporated in statistical models that proposed an immunological basis for epidemiological patterns.³ Due to the implications of that work, the term “immunological imprinting” increased in popularity but has been equivocally applied to describe differential immunological reactivity patterns observed between cohorts with varied exposure histories. However, it is pertinent to note that

what is considered OAS. In some cases, OAS is studied as a bias in baseline antibody titers and, in other cases, as a bias in antibody titers after antigen re-exposure. In the case of the latter, the time of sampling post-re-exposure is also confounding, with earlier and later time points showing different extents of bias. Furthermore, what is considered a “bias” varies greatly, sometimes referring to a difference in antibody affinity toward original versus recall antigens and sometimes referring to a quantitative difference in antibody levels, which can be further confounded by how these are measured (e.g., neutralization versus binding or absorption assays). Yet, in other cases, OAS refers to antibodies recalled *in vivo* by a secondary antigen but do not demonstrate any measurable *in vitro* binding to the same antigen while binding to the original antigen. Over the last few decades, the highly equivocal use of the term “OAS” and its susceptibility to misinterpretation due to connotations of the word “sin” have limited its utility. It might thus be prudent to avoid this term and apply more consistently defined terminology that is less prone to misapprehension.

DEFINING PATTERNS IN IMMUNOLOGICAL REACTIVITY

The term “immunological (or immune) imprinting” is currently used to generically describe the effects of previous exposures on antibody and B cell responses. Yet, it was initially introduced to explain an epidemiological pattern wherein individuals born in similar years (birth cohorts) exhibit lower susceptibility to influ-

not all immunological reactivity differences will translate into clinical or epidemiological differences. Thus, differential susceptibility to infection or disease severity patterns can be considered the “epidemiological imprint” that exposure history leaves on the population level, presumably due to an underlying immunological reactivity pattern (Figure 1A).

In attempting to explain the immunological effects of exposure history, work in the field of immunity to influenza viruses has introduced additional terminology to more specifically describe some of the observed patterns in antibody titers. “Antigenic seniority” refers to a quantitatively defined hierarchy of antibody titers toward related antigens encountered during an individual’s lifetime. In this hierarchy, antigens encountered earlier in life have a more senior position (defined by higher antibody titers against them) than antigens encountered later in life (Figure 1B).¹ It is primarily used in the context of “steady-state” titers from cross-sectional studies. It captures a very similar, if not the same, phenomenon as Francis’ OAS but is more clearly agnostic to any negative connotations. “Back-boosting” refers to increases in antibody titers (hence “boost”) toward antigens encountered previously (hence “back”). Used in longitudinal studies, it describes responses following exposure to antigenically related viruses (like a drifted version of influenza virus following vaccination or re-infection).⁴ The increase in titers due to back-boosting maintains the *absolute* titers toward past antigens at a higher level than *absolute* titers toward newer antigens. However, the *titer boost* (fold difference between pre- and

post-exposure titers) is greater for newer antigens (Figure 1B). Antigenic seniority and back-boosting are manifestations of fundamental immunological processes, namely cross-reactivity and immune memory recall.

IMMUNOLOGICAL PRINCIPLES UNDERLYING THE EFFECTS OF PREVIOUS EXPOSURES

Cross-reactivity is integral to any examination of the immunological effects of repeated exposures to antigenically evolving pathogens. It typically results from the conservation of epitopes between the primary and re-exposure antigens and can have the following effects. First, cross-reactive memory B cells (MBCs) established by previous exposures will be recalled and may either (1) rapidly differentiate into antibody-secreting cells that provide a large pool of antibodies in a matter of days or (2) seed a germinal center reaction that, in the following weeks and/or months, shapes the B cell repertoire. The former will be evident in samples from the acute phase of infection or vaccination, but the latter may only become apparent from samples obtained later. Because the participating MBCs are cross-reactive between previous and re-exposure antigens, antibody titers to both will increase, yielding observations like back-boosting and antigenic seniority.

A second potential effect of cross-reactivity is *antibody feedback*, whereby pre-existing or plasmablast-derived cross-reactive antibodies may block access of B cells directed against the same or adjacent epitopes to the antigen.⁵ Such epitope masking can redirect the focus of the response to other non-masked, and thus available, epitopes. Epitope masking generally requires masked epitopes to be conserved, and thus the subsequent shift in epitope focus may engender reactivity to non-conserved epitopes. It is important to note that in addition to the recall of cross-reactive MBCs, there is evidence that B cells of likely naive origin can participate in such responses to antigen re-exposure. When the latter B cells target non-conserved epitopes, the antibodies produced can expand the breadth of the response. A key question is the extent to which, if any, pre-existing memory (in the form of antibodies or MBCs) may limit *de novo* reactivity to non-conserved epitopes. For example, antibody feedback may also promote antigen clearance, reducing the amount of antigen available to support B cell responses. Also, affinity-matured MBCs may have a competitive advantage for engaging antigens compared to naive B cells. In this way, cross-reactivity and memory recall may focus the response to a limited number of epitopes, which could be detrimental. Evidently, prior exposures' effects on subsequent B cells responses through cross-reactivity and recall are complex. Thus, they cannot be generalized as beneficial or detrimental, nor be considered absolutes. Indeed, impediments imposed by pre-existing memory may be overcome by increasing antigen dose and/or including adjuvants. Such complexity should be considered when contemplating any potential effects of prior exposure.

THE GOOD, THE BAD, AND THE UNKNOWN EPIDEMIOLOGICAL IMPLICATIONS OF PREVIOUS EXPOSURES

The effects of previous exposures have been examined at both the molecular and immunological as well as the clinical and pop-

ulation levels for influenza (see Cobey and Hensley⁶ and Zhang et al.⁷ for detailed discussion). If recall of MBC upon re-exposure focuses antibody responses to a limited number of epitopes, this can increase an individual's susceptibility to infection or disease when these epitopes acquire escape mutations and in the absence of antibodies to other epitopes.⁶ There is also evidence that exposure history may have negatively impacted vaccine effectiveness for influenza A viruses in the last decade.⁸ Such effects appear to be birth-cohort specific, suggesting long-lasting effects of early life exposures, although effects of more recent exposures may also be at play. This exemplifies how imprinting from childhood exposures may have negative consequences. Nonetheless, early life exposure may also exert strong protective effects from the disease. For example, it has been estimated that exposure to H1N1 early in life considerably reduces the risk of medically attended influenza disease from H1N1 and the risk of fatal disease from H5N1 later in life.³ Given the various potential effects of prior exposures, it is important not to generalize imprinting as a barrier to the generation of protective immunity, as is often seen in the literature.

In cases where imprinting by exposure history has been associated with infection or disease outcomes, as described above, immunological data are used to understand the already observed epidemiological patterns. Numerous factors must be considered when attempting the reverse (i.e., extrapolating immunological findings to potential yet unobserved clinical outcomes). Analysis of one group of individuals exposed to strain A and then to strain B (group AB) will typically reveal higher antibodies toward A than toward B (Figure 2A). This is evidence of antigenic seniority but does not constitute evidence that prior exposure to A has a negative effect on antibody responses to B. To determine whether such an interaction exists, the analysis needs to include a group not exposed to A (group B), a comparator frequently missing from the literature. When such a group is included, there are three possible scenarios regarding antibodies against B in group AB relative to group B: first, AB individuals might have the same antibody titers as B individuals. This would indicate no effect of previous exposure to A, despite AB individuals having greater titers to A than to B (antigenic seniority). Second, AB individuals may have higher titers against B than "B only" individuals. This would indicate an immunologically positive effect of previous exposure to A, irrespective of AB individuals having greater titers to A than B. Third, AB individuals may have lower titers against B than "B only" individuals. This would indicate an immunologically negative effect of previous exposure to A. Critically, however, any difference in antibody titers is by itself insufficient to conclude that there will be a difference in protection from infection or disease severity. If one considers the typical shape of a seroprotection curve (Figure 2B), two observations can be noted: (1) there exists an antibody level above which no higher protection is conferred and (2) the same fold difference in antibody titers could have profoundly different effects on protection. Both will depend on the actual antibody levels and the relationship between antibody titers and protection. In addition, protection, especially in the context of disease severity, is generally multifactorial and may not always be distilled to a single immunological measurement. Overall, while immunological data may be useful in formulating hypotheses about protection, caution is warranted in overinterpreting the potential clinical and epidemiological implications of

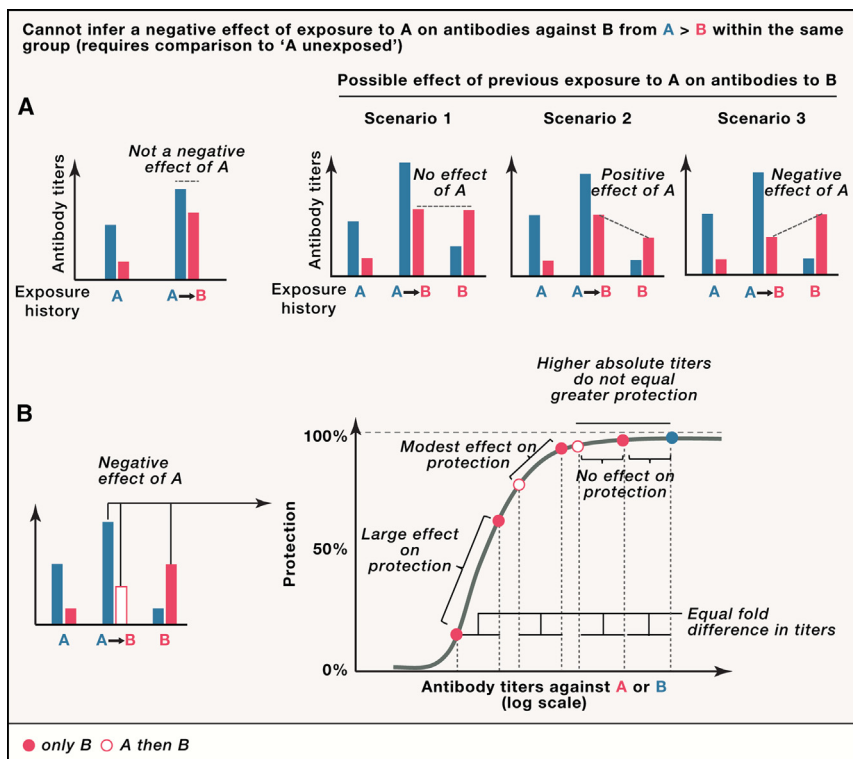


Figure 2. Determining the effects of prior exposures on subsequent immune responses

(A) Inferring any negative effects of previous exposure on subsequent immune responses requires appropriate control groups. Observing greater antibodies toward antigen A than antigen B within an $A \rightarrow B$ group is not sufficient to conclude that prior exposure to A has a negative effect on subsequent response to B.

(B) Differences in antibody titers alone are not sufficient to extrapolate a difference in protection from infection or severe disease. The effects of differential antibody titers will depend on their absolute values and the relationship between antibodies and protection.

exposure history solely from observed differences in antibody reactivity patterns between study groups.

DO PAST EXPOSURES SHAPE IMMUNITY TO SARS-CoV-2?

Immunological imprinting has been considered in many aspects of the humoral immune response to SARS-CoV-2 infection and vaccination. This is typically focused on developing neutralizing antibodies as they are an established correlate of protection. Still, the potential contribution of non-neutralizing antibodies or cellular immunity, which may be less affected by exposure history, should not be disregarded when considering protection from disease. Back-boosting of antibodies cross-reactive to the spike of hCoVs has been observed following SARS-CoV-2 infection and vaccination. However, there is limited evidence to suggest that these antibodies affect the development of neutralizing antibodies against SARS-CoV-2 or that they significantly modulate susceptibility to severe disease.

Perhaps more pertinent is the context of antigenically drifted SARS-CoV-2 variants, in which two relevant observations have been made. First, in individuals primed with an Ancestral SARS-CoV-2 virus (Hu-1-like) (by vaccination or infection) who then become infected with an antigenic variant, neutralizing antibody titers increase against the priming Hu-1-like antigen (back-boosting) as well as the new infecting variant.⁹ Second, individuals primed with Hu-1-like spike and then infected with a variant like Delta or Omicron maintain higher antibodies against the Hu-1-like antigen than the infecting variant (antigenic seniority).¹⁰ As discussed above, both are a manifestation of recall of cross-reactive MBCs established by

Omicron after breakthrough infection,¹¹ which warrants further investigation. Nonetheless, clinical and epidemiological studies support the notion that any prior exposures to SARS-CoV-2 by infection and/or vaccination have a positive impact in mitigating disease severity of subsequent infections.¹² Importantly, at later time points after Omicron breakthrough infection, recalled cross-reactive antibodies exhibit increased affinity and neutralization potency against Omicron,¹³ indicating adaptation of recalled MBCs to the re-exposure antigen.

As booster immunizations with antigenic variants are becoming more common, it is also important to consider how prior exposures may impact the outcomes of such vaccines, at least at the immunological level. A critical question is whether immunization with an Omicron antigen can engender reactivity to non-conserved epitopes or whether memory recall will focus on the immune response to conserved epitopes. Encouragingly, administering bivalent vaccines, including the BA.5 antigen, to previously vaccinated or infected individuals engenders neutralization activity toward more contemporary and antigenically advanced variants.¹⁴ At the molecular level, immunization with an Omicron antigen induces antibodies with *de novo* reactivity patterns toward mutated epitopes,¹⁵ possibly reflecting the recruitment of naive B cells. The existing data suggest that exposure to antigenically drifted SARS-CoV-2 by infection or vaccination successfully broadens immunity either by the recall of cross-reactive memory and/or engendering *de novo* reactivity.

RECAP

Exposure history can affect the subsequent immune response following exposure to antigenically related viruses and

potentially impact protection from disease. Immunological patterns like antigenic seniority and back-boosting can be observed in analyses of antibody reactivity. In contrast, epidemiological patterns may be observed in disease susceptibility between populations with different exposure histories. These effects of prior exposure result from basic immunological processes, namely cross-reactivity and recall of immunological memory. The immunological effects of exposure history need to be considered carefully, using clear and consistent terminology, and caution is urged in extrapolating the effects such immunological differences may have on clinical outcomes. A greater understanding of these immunological phenomena and the underlying mechanism might provide useful insights for developing COVID-19 vaccines against antigenically advanced variants.

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